

## Original Contribution

## Safety of trivalent chromium complexes: No evidence for DNA damage in human HaCaT keratinocytes

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## Abstract

Several studies have demonstrated beneficial effects of supplemental trivalent Cr in subjects with reduced insulin sensitivity with no documented signs of toxicity. However, recent studies have questioned the safety of supplemental trivalent Cr complexes. The objective of this study was to evaluate the cytotoxic and genotoxic potential of the Cr(III) complexes (histidinate, picolinate, and chloride) used as nutrient supplements compared with Cr(VI) dichromate. The cytotoxic and genotoxic effects of the Cr complexes were assessed in human HaCaT keratinocytes. The concentrations of Cr required to decrease cell viability were assessed by determining the ability of a keratinocyte cell line (HaCaT) to reduce tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. DNA damage using the Comet assay and the production of 8-hydroxy-2'-deoxyguanosine were also determined with and without hydrogen peroxide-induced stress. The LC50 for human cultured HaCaT keratinocytes was 50  $\mu$ M for hexavalent sodium dichromate and more than 120-fold higher for Cr chloride (6 mM) and Cr histidinate (10 mM). For Cr picolinate at saturating concentration (120  $\mu$ M) the LC50 was not attained. High Cr(III) concentrations, 250  $\mu$ M Cr as Cr chloride and Cr histidinate and 120  $\mu$ M Cr picolinate (highest amount soluble in the system), not only did not result in oxidative DNA damage but exhibited protective antioxidant effects when cells were exposed to hydrogen peroxide-induced oxidative stress. These data further support the low toxicity of trivalent Cr complexes used in nutrient supplements.

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Naturally occurring trivalent Cr (Cr(III)) can be converted to the hexavalent form (Cr(VI)) at high pH and temperature. Hexavalent Cr is a well-documented toxin, mutagen, and carcinogen [18]. Insoluble hexavalent Cr compounds induce anchorage independence and cytotoxicity in cultured diploid human fibroblasts and morphological (focus formation), anchorage-independent, and neoplastic transformation in C3H/10T1/2 mouse embryo fibroblasts [30]. The hexavalent compounds are more than 1000-fold more cytotoxic and mutagenic than the Cr(III) compounds in normal diploid human fibroblasts [12].

Cr(VI) induces oxidative stress, DNA damage, apoptotic cell death, and altered gene expression [29]. In contrast, trivalent Cr potentiates insulin action and improves normal lipid and carbohydrate metabolism [3,4,16]. Western diets are often low in Cr(III) and numerous studies have shown beneficial effects of supplemental Cr on glucose, insulin, lipids, and related variables of subjects with glucose values ranging from slightly elevated to diabetic [1,3,4,16]. Chromium is a nutrient and not a drug; therefore only subjects whose impaired glucose, insulin, lipid, and related abnormalities are related to insufficient dietary Cr will be improved by additional Cr [3].

Several dietary Cr(III) supplements are currently available to alleviate this deficiency and they are widely used because interventional trials have demonstrated the beneficial effects of Cr(III) supplementation on insulin sensitivity, blood lipids, and

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related abnormalities and potential effects in reducing weight [2,31]. The effects of supplemental Cr on weight reduction are controversial [45]. Most studies that do not report effects on weight or lean body mass are short term (less than 12 weeks); involve healthy, young normal subjects with good insulin sensitivity; and also involve 200 µg or less of supplemental Cr [2]. A recent well-controlled study involving subjects with type 2 diabetes mellitus (DM) on sulfonylurea therapy documented effects of supplemental Cr(III) on attenuated body weight gain and visceral fat accumulation in addition to improvements in insulin sensitivity and glucose control [28].

When Cr(VI) enters the cells it is reduced to Cr(III) and the resulting trivalent Cr can attack cellular components, including DNA. However, naturally occurring Cr(III) has a weak ability to enter the cell and only very low levels of Cr(III) enter the nucleus. Despite this, there are reports of DNA-damaging effects of Cr(III) both in cellular and in cell-free systems. The redox potential of Cr has been implicated in the generation of oxidative damage causing deleterious DNA mutations [40,42,45]. The deleterious interaction of Cr(III) with DNA is supported by direct Cr–DNA binding [26,47]. The possible carcinogenic mechanisms of Cr(III) might also be related to its ability to generate hydroxyl radicals ( $\cdot\text{OH}$ ) via a Fenton reaction [44]. It has been reported that Cr(III) picolinate causes chromosomal and DNA damage and some of these papers reported that the picolinate moiety may be responsible for this toxicity [38,40].

The objective of this study was to determine whether the trivalent Cr complexes used as supplements and pharmacological treatments for metabolic syndrome and type 2 DM are safe by determining the cytotoxic and genotoxic potential of the Cr(III) complexes (histidinate, picolinate, and chloride) compared with Cr(VI) dichromate. DNA damage in human HaCaT keratinocytes with and without  $\text{H}_2\text{O}_2$ -induced oxidative stress was evaluated.

## Materials and methods

### Reagents

The cell medium RPMI 1640 was obtained from Invitrogen (Cergy Pontoise, France), fetal calf serum was from ATGC Biotechnologie (Noisy-le-Grand, France), penicillin and streptomycin were from Roche Diagnostic (France), and sodium dichromate was from Aldrich Chemical Co. (Milwaukee, WI, USA). All chemical compounds were of analytical grade or higher.

### Cell culture and chromium complexes

HaCaT keratinocytes ( $2 \times 10^5$  cells/35-mm petri dish) were incubated for 24 h at 37°C in RPMI 1640 medium supplemented with 10% fetal calf serum containing penicillin (1.25 U/ml) and streptomycin (0.00125 U/ml) with varying concentrations of the forms of Cr evaluated in this study (histidinate, picolinate, and chloride). Synthesis and absorption of the Cr(III) complexes have been described [6]. The

chromium histidine complex contained 3 mol of histidine per mole of chromium and the binding of the Cr to the imidazole nitrogen was confirmed using proton and  $^{13}\text{C}$  NMR. Chromium–histidine complex is covered by U.S. Patent 6,689,383, Chromium–histidine complexes as nutritional supplements, and European Patent Application 00970641.7/1235835.

### Cell viability (MTT assay)

The concentrations of Cr(III) required to induce a decrease in cell viability were assessed by determining the ability of the HaCaT cells to reduce the tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The assay is based on cleavage of the tetrazolium salt, MTT, by mitochondrial succinate dehydrogenase to form a colored soluble formazan complex which is quantified spectrophotometrically at 570 nm. The keratinocyte suspension was seeded in 96-well culture plates using 100 µl of medium (20,000 cells) per well. The next day, varying amounts of the Cr complexes were added to the wells and the culture plates were incubated for 24 h at 37°C. For the MTT assay, 10 µl MTT (5 mg/ml stock in PBS) was added to each well and cells were incubated at 37°C for 2 h. Viable cells convert the soluble yellow MTT to insoluble purple formazan by the action of mitochondrial succinate dehydrogenase. One hundred microliters of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The optical density was measured at 570 nm with a microplate reader (Multiskan, Labsystems) and the amount of formazan produced was calculated by using the molar extinction coefficient of 51,000. The absorbance is proportional to viable cell number and survival was calculated as the percentage of the staining values of untreated cultures.

### Lactate dehydrogenase (LDH) release

The release of LDH is a marker of cell necrosis, and the activity of LDH is correlated with cell death. LDH activity was measured using Sigma Diagnostics LDH Kit 500 (St. Louis, MO, USA).

### DNA damage assessed by the comet assay

The Comet assay was performed according to the procedure of Sing et al. [36] modified by Hininger et al. [21]. Ten microliters containing roughly 20,000 cells was mixed with 110 µl of 0.6% low-melting-point agarose in RPMI 1640 at 37°C. One hundred ten microliters of the mixture was layered onto a slide precoated with thin layers of 1% agarose and immediately covered with a cover glass. Slides were left for 10 min on ice to allow the agarose to solidify. After the cover glass was gently removed, the slides were immediately immersed into an ice-cold freshly prepared lysis solution (2.5 M NaCl, 10 mM  $\text{Na}_2\text{EDTA}$ , 10 mM Tris hydroxymethyl aminomethane, 1% sodium sarcosinate, 1% Triton 100-X, and 10% DMSO, pH 10) to lyse the cells and to allow DNA unfolding. After 1 h in the dark at 4°C, the slides were immersed

for 25 min in a fresh alkaline electrophoretic buffer (300 mM NaOH, 200 mM Na<sub>2</sub> EDTA, pH 13) for unwinding and then separated by electrophoresis (25 V/300 mA, 25 min). All the steps were carried out under minimal illumination. The electrophoresis tank was covered with black paper to avoid any additional light-induced DNA damage. Once electrophoresis was completed, slides were neutralized (3 × 5 min; 0.4 M Tris, pH 7.5). The dried microscope slides were stained with ethidium bromide (20 µg/ml in distilled H<sub>2</sub>O; 50 µl/slide), covered with a coverslip before being analyzed using a Zeiss fluorescence microscope under green light. Results were expressed as the percentage of DNA in the tail (% tail). Fifty images were randomly selected from each sample, and the size of the tail, which corresponds to the increased DNA damage, was measured. Three parallel tests with aliquots of the same sample of cells were performed for a total of 150 cells, and the % mean tail was calculated.

#### Measurement of oxidized bases (8-oxo-deoxyguanosine)

After cell membrane disruption, DNA was extracted at pH 8 in the presence of 100 µM desferoxamine solution with NaI, 4 mM, and isopropanol; washed once with 2 ml isopropanol and once with 2 ml 70% ethanol; and dissolved in 175 µl of desferoxamine solution.

DNA was hydrolyzed for 1 h with 10 U of nuclease P1 at 37°C. Nucleotides were dephosphorylated using 8 U of alkaline phosphatase for 1 h at 37°C. Proteins were removed by ultrafiltration with the MPS-1 kits. The nucleoside solution was then analyzed by HPLC and coulometric detection using the general methods described by the ESCODD group [20].

8-Oxo-deoxyguanosine (8-OHdG) production was measured in the column eluent by coulometry with a 190-mV potential applied on the first electrode and a 310-mV potential on the second electrode and detected by spectrophotometry at 280 nm. The mobile phase consisted of 2 liters of a 50 mM, pH 5.5, phosphate buffer containing 12% methanol, and HPLC separation was run at 26°C. Under these conditions, 8-OHdG was eluted at 12 min and desoxyguanosine (dG) eluted at 9 min. The data are expressed as the percentage of 8-OHdG/10<sup>5</sup> dG compared with control.

#### H<sub>2</sub>O<sub>2</sub>-induced oxidative stress

The cells were subjected to hydrogen peroxide (50 µM) for 30 min at 37°C after a 24-h preincubation period with concentrations of Cr(III) at 5, 50, and 250 µM. Each experiment included a control group of cells exposed to H<sub>2</sub>O<sub>2</sub> at 50 µM without Cr preincubation.

#### Statistical methods

The data are expressed as means ± SD. The differences between means were evaluated by applying Student's *t* test and ANOVA. The level of statistical significance was set at *p* < 0.05. The data were analyzed using Statistica Package (StatSoft Software, Paris, France).

## Results

### Evaluation of DNA damage at LC50

#### Cell viability

Cell viability, expressed as a percentage of cell survival in the presence of Cr(III) compounds and sodium dichromate, is presented in Fig. 1. The LC50, evaluated by MTT test, was 50 µM for hexavalent sodium dichromate and more than 120-fold higher for Cr chloride (6 mM) and Cr histidinate (10 mM). For Cr picolinate, at saturating concentration (120 µM), LC50 was not attained.

#### Cell necrosis

There was no significant cell necrosis assessed by LDH release when keratinocytes were incubated with Cr chloride (6 mM) and Cr histidine (10 mM) at LC50.

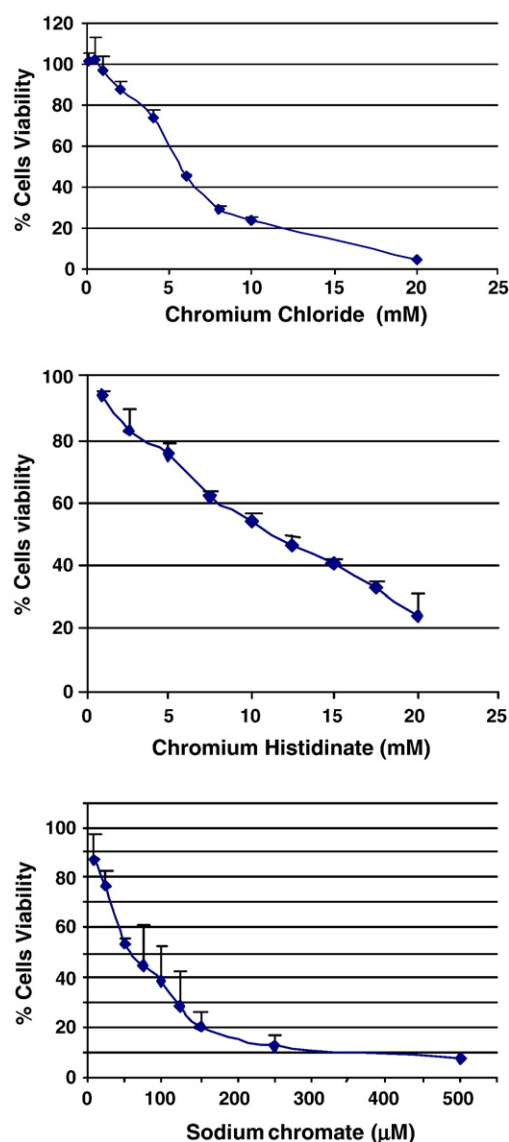


Fig. 1. Viability of HaCaT keratinocytes in the presence of Cr(III) compounds and sodium dichromate (Cr(VI)).

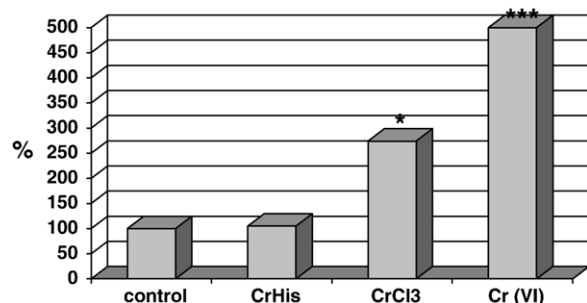


Fig. 2. Percentage DNA damage in HaCaT keratinocytes in the presence of Cr(VI) and Cr(III) compounds at LC50. Human HaCaT keratinocytes were exposed for 24 h at 37°C to Cr(III) histidinate (10 mM), Cr(III) chloride (6 mM), or sodium dichromate (50  $\mu$ M). The number of cells in each treatment was 150. Data are expressed as DNA damage assessed by Comet assay, with control as 100%. \* $p$  < 0.05, \*\*\* $p$  < 0.001.

### DNA damage

Cell exposure to sodium dichromate and Cr chloride at LC50 led to a significant increase, 500 and 250% increase of tail extent, respectively, in DNA damage as assessed by Comet assay in comparison with control cells (Fig. 2), whereas there were no oxidative DNA alterations in cells exposed to Cr histidinate (Fig. 2, Table 1) as assessed using both the Comet assay and 8-OHdG production. A significant increase in oxidized bases as 8-OHdG was observed with sodium dichromate and Cr chloride, and no detected changes were seen with Cr histidinate (Fig. 3).

### Evaluation of DNA damage at the highest noncytotoxic concentrations of Cr(III)

Using the MTT test, we determined the highest doses that did not result in cytotoxic effects: 250  $\mu$ M for Cr chloride; 250  $\mu$ M for Cr histidinate, and 120  $\mu$ M for Cr picolinate. As shown in Table 2, after 24 h incubation with Cr chloride (250  $\mu$ M), Cr histidinate (250  $\mu$ M), and Cr picolinate (120  $\mu$ M), these high metal concentrations did not cause DNA damage as assessed by the Comet assay (Table 2) and, in parallel, we observed a decrease in 8-OHdG of 30% or more ( $p$  < 0.01) (Fig. 4).

### Evaluation of DNA damage in cells subjected to H<sub>2</sub>O<sub>2</sub>-induced stress

In cells preincubated for 24 h with Cr(III) forms and then subjected to H<sub>2</sub>O<sub>2</sub>-induced oxidative stress, there was no

Table 1  
DNA damage in HaCaT keratinocytes in the presence of Cr(VI) and Cr(III) forms at LC50

	% Tail
Control	2.12±0.58
Cr histidinate (10 mM)	2.13±0.34
Cr chloride (6 mM)	5.68±1.68*
Sodium dichromate (50 $\mu$ M)	> 12***

Each treatment was repeated in three separate assays. The number of cells in each treatment was 150. Data are expressed as % tail ± SD assessed by Comet assay.

\*  $p$  < 0.05.

\*\*\*  $p$  < 0.001.

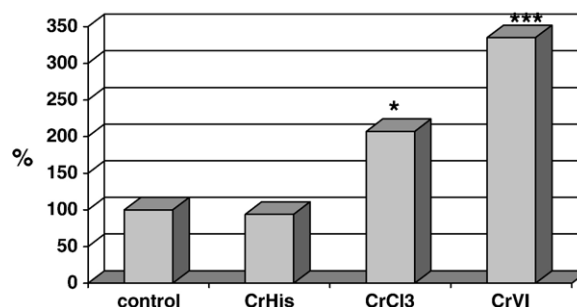


Fig. 3. 8-OHdG production in HaCaT keratinocytes in the presence of Cr(III) and Cr(VI) LC50 concentrations. Human HaCaT keratinocytes were exposed for 24 h at 37°C to Cr(III) histidinate (10 mM), Cr(III) chloride (6 mM), or sodium dichromate (50  $\mu$ M). The number of cells in each treatment was 150. Data are expressed as DNA damage assessed by Comet assay, with control as 100%. \* $p$  < 0.05, \*\*\* $p$  < 0.001.

evidence for pro-oxidant effects of the forms of Cr tested. In contrast, we observed significant protective effects against DNA oxidative damage monitored by a decrease in % tail moment when cells were preincubated with Cr histidinate, Cr picolinate, or Cr chloride at 50 and 5  $\mu$ M (Table 3).

### Discussion

Skin cells, HaCaT human keratinocytes, were used as a model for Cr toxicity because Cr salts have been shown to induce skin cancer and contact allergic dermatitis [22]. These cells have been used as a suitable model for chromium, nickel, and cobalt toxicity studies and these cells concentrate metals, including chromium, from the medium, leading to potential increases in sensitivity to metals [19]. Lymphocytes [10,11], bacterial cells [32], and dermal fibroblasts [35] have also been used to study the genotoxicity and cytotoxicity of chromium.

Reduction of Cr(VI) is associated with free radical reactions and plays an important role in Cr(VI)-induced carcinogenesis [14]. In human HaCaT keratinocytes, we confirmed that sodium dichromate was highly genotoxic with increased DNA damage evaluated by Comet assay, a sensitive method to assess DNA damage [27], and increased 8-OHdG production, a good marker of oxidative stress [34]. In comparison, Cr(III) compounds such as chloride and histidinate were 120- and 200-fold less toxic, respectively. The range for LC50 toxicity was NaCr (VI) (50  $\mu$ M) > Cr chloride (6 mM) > Cr histidinate (10 mM). These concentrations are in the same range as those

Table 2  
No DNA damage in HaCaT keratinocytes in the presence of Cr(III) forms at the highest noncytotoxic doses

	% Tail
Control	2.05±0.55
Cr histidinate (250 $\mu$ M)	1.97±0.31
Cr chloride (250 $\mu$ M)	1.93±0.32
Cr picolinate (120 $\mu$ M) (saturated solution)	1.50±0.18 ( $p$ < 0.07)

Each treatment was repeated in three separate assays. The number of cells in each treatment was 150. Data are expressed as % tail ± SD assessed by Comet assay.



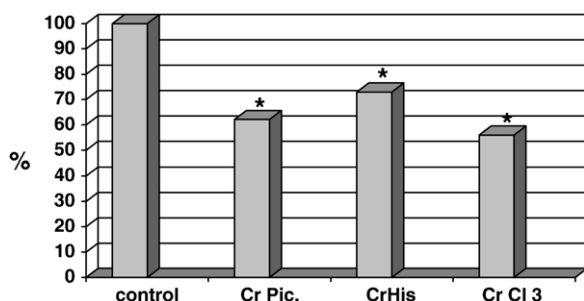


Fig. 4. 8-OHdG production in HaCaT keratinocytes in the presence of Cr(III) forms at the highest noncytotoxic doses. Each treatment was repeated in three separate assays. In each assay, the number of cells was 150 per treatment. Concentrations: Cr picolinate 120  $\mu$ M, Cr histidine 250  $\mu$ M, Cr chloride 250  $\mu$ M. Data are expressed as percentage of 8-OHdG production, with control group as 100%. \* $p < 0.05$ .

studied in human HaCaT keratinocytes [19] with EC<sub>50</sub> (24 h) values of 30  $\mu$ M for Cr(VI) and Cr chloride not toxic up to 1 mM. In our study, the toxicity at LC<sub>50</sub> is not associated with an increase in LDH release, a marker of necrosis. Despite the toxicity of Cr(III) as chloride being very low, this toxicity was reported higher than that of picolinate in human fibroblast studies [35]. In cell-free systems, caspase-3 activation has also been described as possibly involved in Cr chloride-induced apoptosis [10].

Even though the toxicity of Cr picolinate has never been demonstrated in humans, the safety of this form has been questioned [45] and extensively examined for toxicity regarding its ability to generate oxidative stress and being mutagenic. In the present work, we observed that Cr picolinate at 125  $\mu$ M (saturating solution) exhibited the least toxicity in decreasing the survival rate of the cells (data not shown), compared with Cr chloride and Cr histidine. Our observation is in agreement with those of Shrivastava et al. [35] in fibroblasts that showed a lower decrease in cell viability with Cr picolinate than with Cr chloride. Most studies of genotoxicity in cellular systems have been negative for trivalent Cr. However, other studies suggest that Cr picolinate may cause oxidative DNA damage and therefore could be mutagenic. Cr(III) picolinate has been reported to generate increased hydroxyl radicals [9], cleave DNA [38], induce apoptosis of lymphocytes [11], and alter DNA topology [32]. It has been suggested that the potential intracellular accumulation of Cr(III) could be a leading factor in the formation of bonds between Cr(III) and DNA, causing genotoxic effects. However, most of these studies were done in cell-free systems using DNA purified from biological materials, whereas it is generally considered that only small amounts of Cr(III) compounds are absorbed and enter cells in animals. The absorption of Cr in animals is very low, usually less than 2% [2,6]; therefore the nucleus of the cell is not exposed to high levels of Cr. Recently, mutagenicity of chromium picolinate and chloride was studied in *Salmonella typhimurium* and L5178Y lymphoma cells [46]. Neither chromium picolinate nor chromium chloride induced a mutagenic response in *S. typhimurium*. In contrast, in the L5178Y mouse lymphoma mutation assay, chromium picolinate induced a mutagenic response at relatively nontoxic dose levels. Whereas lympho-

cytes exposed to Cr(VI) displayed extensive DNA damage, such an effect was not observed when Cr(III) was tested and the Comet assay did not indicate the involvement of oxidative mechanisms in the DNA-damaging activity of trivalent Cr [14].

In J774A.1 macrophages, Bagchi et al. [8] reported low levels of oxidative stress, low (1.2- to 1.3-fold) increases in DNA fragmentation, and no significant loss in cell viability with 30 to 50  $\mu$ g/ml concentrations of Cr picolinate. In parallel, a characterization of nonmutagenic Cr(III)–DNA interactions has been published [13]. In animals, data are also controversial. In rats, the lack of toxicity of Cr chloride and Cr picolinate has been established [5]. Similarly, Cr chloride and Cr niacin, used also as nutritional supplements, were unlikely to produce oxidative damage in animals [9]. In contrast, in hamster cells in culture, Cr picolinate was reported to be mutagenic and induced morphological damage [41]. It was suggested that the coordination of Cr(III) with picolinic acid may make the metal more genotoxic than other forms of Cr(III). However, these data are now contested because another recent study did not find a mutagenic effect of Cr picolinate using the same hamster ovary cell model [37].

In obese subjects supplemented with 400  $\mu$ g/day of Cr as Cr picolinate, Kato et al. [25] did not observe increased oxidative DNA damage as measured by anti-HMdU antibody levels.

Regarding Cr histidine, which is absorbed better than other available forms of Cr [6], there was no DNA damage at the LC<sub>50</sub> as assessed using the Comet assay and 8-OHdG production. At this dose, an arrest of DNA synthesis could be suggested, given that nonmutagenic Cr(III)–DNA interactions have been shown to arrest DNA synthesis [13,15].

The level at which trivalent Cr could become potentially harmful is, however, still an unanswered question. In this study, using the MTT test of cell viability, we determined the highest noncytotoxic concentrations of Cr chloride, Cr histidine, and Cr picolinate. At these doses, we observed a trend of the Comet tail moment to decrease and, for all the forms, a significant ( $p < 0.01$ ) decrease in 8-OHdG production. These data suggest an antioxidant effect of Cr(III) forms. They are in agreement with the study of Jain et al. [23] showing that

Table 3

DNA damage in cells preincubated with Cr(III) forms at various concentrations and exposed to H<sub>2</sub>O<sub>2</sub>-induced oxidative stress

	Tail %
Control	
H <sub>2</sub> O <sub>2</sub> -induced oxidative stress	3.60±0.10
Plus Cr histidine (250 $\mu$ M)	3.59±0.04
Plus Cr histidine (50 $\mu$ M)	2.70±0.25*
Plus Cr histidine (5 $\mu$ M)	2.90±0.35*
Plus Cr chloride (250 $\mu$ M)	3.04±0.19
Plus Cr chloride (50 $\mu$ M)	2.60±0.14*
Plus Cr chloride (5 $\mu$ M)	2.75±0.15*
Plus Cr picolinate (120 $\mu$ M)	3.59±0.10
Plus Cr picolinate (50 $\mu$ M)	2.90±0.25*
Plus Cr picolinate (5 $\mu$ M)	2.90±0.15*

Each treatment was repeated in three separate assays. The number of cells in each treatment was 150. The data are presented as % tail±SD.

\*  $p < 0.05$ .

Cr(III) chloride inhibited oxidative stress and decreased lipid peroxidation and TNF- $\alpha$  secretion in cultured U937 monocytes. In hepatocytes, Tezuka et al. [43] also demonstrated that Cr(III) chloride inhibited oxidative stress and decreased lipid peroxidation.

In humans, our group also reported antioxidant effects of Cr(III) supplementation in a double-blind clinical study involving people with type 2 diabetes [7]. These antioxidant effects were also confirmed by another study using 600  $\mu\text{g/day}$  Cr from yeast in people with type 2 diabetes [17].

This study was also designed to investigate the effects of Cr(III) compounds on DNA damage in the presence of  $\text{H}_2\text{O}_2$  (50  $\mu\text{M}$ )-induced stress resulting in a 50% increase in DNA damage by Comet assay. After 24 h preincubation of the cells with the Cr(III) compounds, there was a significant protective effect on DNA damage for all forms of Cr(III) at doses below 100  $\mu\text{M}$  and no deleterious effects at 250  $\mu\text{M}$ . The dose range of Cr(III) showing protective effects was 50 and 5  $\mu\text{M}$ . These doses are comparable to the amounts humans are postulated to absorb in long-term daily supplementation with 200  $\mu\text{g}$  of Cr as picolinate [39]. At these doses, we confirmed antioxidant protective effects of Cr(III) complexes against  $\text{H}_2\text{O}_2$ . These effects have been reported on protein oxidation and lipid peroxidation in a human promonocytic cell line [24]. In contrast to our data, another study [33] reported Cr(III)/ $\text{H}_2\text{O}_2$ -induced DNA damage, and a Cr(III)-mediated Fenton-like reaction has been invoked. This notion can be refuted because such a mechanism of DNA damage occurs only at supraphysiological doses of  $\text{H}_2\text{O}_2$  and there is no evidence that this is a viable mechanism at environmental/nutritional concentrations of Cr(III) and physiological amounts of  $\text{H}_2\text{O}_2$ .

## Conclusion

We did not detect significantly increased DNA breakage or increased levels of 8-OHdG in Cr(III)-treated cells and our data do not support the genotoxicity of dietary Cr(III) complexes used as dietary supplements. We demonstrated that high Cr(III) concentrations (250  $\mu\text{M}$  Cr chloride and Cr histidinate and 120  $\mu\text{M}$  Cr picolinate) not only did not result in oxidative DNA damage but rather resulted in antioxidant effects. When cells were exposed to  $\text{H}_2\text{O}_2$ -induced stress, no oxidative damage was observed even at high Cr(III) concentrations (250  $\mu\text{M}$ ). At lower concentrations, below 100  $\mu\text{M}$ , which could be potentially found in nutritional supplementation, we showed beneficial antioxidant effects of all the studied Cr(III) compounds (chloride, histidinate, and picolinate) in protecting DNA from oxidative damage. This study does not support a lack of Cr(III) toxicity but rather low toxicity—lower than that observed for most nutrients.

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patent is a U.S. Department of Agriculture patent with Richard A. Anderson, Marilyn M. Polansky, and Noella Bryden.

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